

# Preparation and characterisation of whey protein fluid gels

Moakes, R.J.A.; Sullo, A.; Norton, Ian

DOI:

[10.1016/j.foodhyd.2014.11.024](https://doi.org/10.1016/j.foodhyd.2014.11.024)

License:

Creative Commons: Attribution (CC BY)

*Document Version*

Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*

Moakes, RJA, Sullo, A & Norton, I 2015, 'Preparation and characterisation of whey protein fluid gels: the effects of shear and thermal history', *Food Hydrocolloids*, vol. 45, pp. 227-235.  
<https://doi.org/10.1016/j.foodhyd.2014.11.024>

[Link to publication on Research at Birmingham portal](#)

## **Publisher Rights Statement:**

2014 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/3.0/>).

## **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

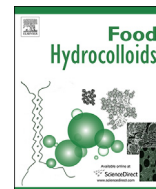
Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.



# Preparation and characterisation of whey protein fluid gels: The effects of shear and thermal history



R.J.A. Moakes<sup>\*</sup>, A. Sullo, I.T. Norton

Centre for Formulation Engineering, School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

## ARTICLE INFO

### Article history:

Received 8 July 2014

Accepted 24 November 2014

Available online 4 December 2014

### Keywords:

Whey protein isolate/WPI

Fluid/shear gels

Particulate gels

Thermal history

Shear history

Rheology

## ABSTRACT

With the potential use of fluid gels in structuring fluids, whey protein isolate (WPI) was used as a prerequisite to prepare fluid gels: owing to its high nutritional value and thermal stability on gelling. Fluid gels were prepared via heat-induced gelation of a 10 wt% protein solution under controlled temperature and shear. Physical properties of the resulting gel particles (e.g. size and particle–particle interactions) were found to be dependent on the combination of shear and thermal history. Discrete large aggregates ( $>120\ \mu\text{m}$ ) were obtained at low shear, with aggregate size decreasing ( $<40\ \mu\text{m}$ ) at higher shear. Such microstructural changes in the particles led to the control of the suspension rheology. All suspensions showed a marked shear thinning behaviour associated with particle break-up which was observed to be more apparent for larger aggregates, originally made at low shear. The viscoelastic properties of the particulate systems, once in intimate contact (e.g. high volume fraction) resembled a pseudo solid material. In addition, it was shown that at a given volume fraction, the elasticity of the suspension varied dependant on their original processing conditions, owing to the degree of particle–particle interactions. A qualitative model has therefore been presented for the formation of particles by which the rheology is determined.

© 2014 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/3.0/>).

## 1. Introduction

Particulate gel suspensions with particles in the micron-scale have received increasing interest in recent years, especially within the food industry where they have been applied to structure fluids (Fernández Farrés, Moakes, & Norton, 2014; Sullo, Watson, & Norton, 2014). Further to this their textural role as fat mimetics has been recently proposed, as the presence of small, soft and smooth particles lowers the friction between oral surfaces providing enhanced fat perception (Fernández Farrés, Douaire, & Norton, 2013). Additionally, it was shown that the rheology of full fat mayonnaise could be simulated by replacing the majority of oil droplet content with spherical agar gel particles (J. E. Norton & Norton, 2010).

An essential feature regarding their application is the novel rheological properties of fluid gels, since they behave in a solid-like fashion at rest, but flow above a critical value of applied stress (I. T. Norton, Jarvis, & Foster, 1999). The rheological properties can be finely controlled to meet requirements for desired applications by

varying the composition (e.g. polysaccharide, protein), cross-link density (e.g. salt, concentration), particle size and/or degree of particle–particle interactions. Both particle size and degree of interactions are directly related to the processing conditions used in production (Gabriele, Spyropoulos, & Norton, 2009; Garrec & Norton, 2012).

Fluid gels are formed when a separation process is applied to a biopolymer system undergoing its sol–gel transition (I. T. Norton et al., 1999). Amongst all the existing methods for the preparation of gel particles (e.g. emulsion route or biopolymer mixtures), shear induced fluid gels are typically more attractive due to relatively easy modulation of resulting properties, by varying two parameters: shear and thermal history. On the other hand, particles produced via a phase separation route (e.g. emulsion route) require additional steps; due to the transfer of produced particles into an aqueous phase, lowering efficiency.

The mechanism proposed for the formation of shear gels is based primarily on nucleation and growth (I. T. Norton et al., 1999). It has been proposed that thermodynamically driven spherical gel particles form mainly through growth of the initial gel nuclei via enrichment, when the applied shear is comparable to the time taken for polymer to diffuse through the system and order (Garrec, Guthrie, & Norton, 2013). Thus the rate of ordering is key to particle

<sup>\*</sup> Corresponding author.

E-mail address: [rjm116@bham.ac.uk](mailto:rjm116@bham.ac.uk) (R.J.A. Moakes).

growth. Rapid ordering forces particle growth through particle–particle structuring; this causes particles to rapidly reach sizes that become affected by the applied shear forces. Hence particle size becomes determined by the applied mechanical break-up and large ( $>100\ \mu\text{m}$ ) anisotropic particles are formed (Gabriele, Spyropoulos, & Norton, 2010).

Such changes in the microstructure led to varying flow properties, which fall in between the behaviour of colloidal particles and polymeric gels (Altmann, Cooper-White, Dunstan, & Stokes, 2004; Gabriele et al., 2010; Garrec et al., 2013). At low volume fractions ( $\phi \sim 0.2$ ) polysaccharide fluid gels showed high viscosities and strong shear thinning behaviour as expected for highly aggregated suspensions, mainly dominated by colloidal forces. The prevention of a continuous gel network in this way leads to incomplete gelation at the particle surface. As a result, significant particle–particle interactions can occur, reported to give rise to both yield stresses and subsequent shear thinning behaviour (Berli & Quemada, 2000; Garrec et al., 2013).

At high phase volumes where particles are in contact and so their movement becomes sterically confined, the fluid gels behave as a suspension of soft particles with rheology dependant on particle elastic modulus as well as particle–particle interactions (Fernández Farrés et al., 2013; Gabriele et al., 2009). The deformability of such soft gel particles, particularly at higher concentrations has been suggested as an explanation for effective volumes exceeding those characteristic of hard sphere suspensions (Adams, Frith, & Stokes, 2004).

Microparticulated whey protein (MWP), as a result of thermal/shear treatment has been shown to change surface and colloidal properties (Dissanayake & Vasiljevic, 2009). Such particulates have modified foaming and emulsifying activity, however further heating formed gels with much lower gelling temperatures and significantly higher complex moduli (Dissanayake, Kelly, & Vasiljevic, 2010; McClements & Keogh, 1995; Nicolai, Britten, & Schmitt, 2011). Continued processing of the MWP also enhanced flow characteristics through increased uptake of the continuous phase, as aggregate size increased (Chung, Degner, & McClements, 2014). Such increased structural properties have been shown to augment textural and sensory perceptions of dairy products (Meza, Verdini, & Rubiolo, 2010; Torres, Janhøj, Mikkelsen, & Ipsen, 2011).

Fat replacers based on biopolymer particles with controlled size and shape are already commercially available. *Simplesse*<sup>®</sup> is a system of microparticulated, denatured protein spheres between 0.1 and 3  $\mu\text{m}$  in diameter; owing to their size and morphology the protein are able to replicate organoleptic mouth feel. The protein particles are formed using a rapid heating process (up to 120 °C) over a short period of time to denature the whey protein. Reducing the pH of the system below the isoelectric point increasing electrostatic repulsion, aided by aggregate blocking agents (i.e. lecithin) and high shear, led to an incomplete aggregation process resulting in discrete particles (Singer, Yamamoto, & Latella, 1988).

From an application point of view, coupled with the highly controllable rheological properties, thermal stability and formulation simplicity are equally important within industry: owing to many potential thermal processes and cost effectiveness within product production. This study therefore investigates the formation of thermally stable whey protein aggregates by applying shear gel technology to a gelling system. The research uses whey protein isolate (WPI), owing to its irreversible heat induced gelation, as a potential candidate for the preparation of fluid gels without additives. Therefore, the production of WPI fluid gels at native pH are investigated, with emphasis to the effect of shear and thermal history on particle intrinsic properties, and corresponding rheological properties thereafter.

## 2. Materials and methods

### 2.1. Materials

Whey protein isolate (WPI) (WPI, W994, S-493391) was obtained from Kerry Ingredients (Listowel, Ireland). The composition of the WPI as stated by the supplier was 91.0% protein, moisture 4.0%, fat 1.0%, ash 3.5% and lactose 0.5%. Mineral content of the WPI was: Ca – 0.50, P – 0.65, Na – 0.10, K – 0.15, Mg – 0.02 and Cl – 0.02%.

### 2.2. Preparation of stock solutions

Whey protein stock solutions of 10 wt% were prepared by dispersing WPI powder in deionised water. Sodium Azide (0.02 wt %) was added to the solution to prevent bacterial growth. Solutions were then stirred for two hours at ambient temperature until completely dispersed and stored at 5 °C until usage (pH remained unadjusted ca. pH 6.4).

### 2.3. Fluid gel preparation using a rheometer

A Malvern Kinexus Pro, stress controlled rheometer (Malvern Instruments Ltd, UK) equipped with a couette measuring system, cup diameter 27 mm and vane diameter 25 mm was used to prepare all WPI fluid gels. Aliquots of WPI solution were transferred using a syringe to the rheometer cup set to a temperature of 25 °C. The geometry was then lowered and sample temperature left to equilibrate for 2 min before shear-heating profiles were undertaken. Heating profiles starting at 25 °C and increasing to 80 °C with a 10 min holding period were conducted at various heating rates (1, 3, 5 and 10 °C min<sup>-1</sup>) under steady shear (200, 400, 600 and 800 s<sup>-1</sup>) before quiescent cooling at 3 °C min<sup>-1</sup> to 20 °C. In all experiments a thermal cover was placed over the system to help prevent water loss. Samples were then removed from the geometry and stored at 5 °C for 24 h before being prepared into known volume fractions and further tests carried out (as described in Section 2.7). Each sample was prepared in triplicate with apparent viscosity profiles showing the average of at least three repeats.

### 2.4. Static light scattering (SLS)

Particle size distributions were determined using static light scattering. A Malvern Mastersizer MS2000 (Malvern Instruments Ltd, UK) attached to a Hydro SM manual small volume sample dispersion unit was used to obtain both surface and volume weighted means,  $d_{3,2}$  and  $d_{4,3}$  respectfully. Particle sizes are calculated based on the Mie theory as such particles are assumed to be monodisperse homogeneous spheres. Samples were prepared by diluting gel particles in distilled water (RI = 1.33) to avoid multiple scattering. Average distributions based on 3 runs were then taken in triplicate.

### 2.5. Optical light microscopy

An optical light microscope, Brunel SP300-fl (Brunel Microscopes Ltd, UK) fitted with an SLR camera (Canon EOS Rebel XS, DS126 191) was used to image fluid gel particles with objective lenses up to 40 $\times$  magnification (5 $\times$ , 10 $\times$ , 20 $\times$  and 40 $\times$ ). Slides were prepared by firstly diluting fluid gel samples (1:4), then adding one drop onto a microscope slide (VWR, UK) and covered with a coverslip (Thickness No.1, VWR, UK).

## 2.6. Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) samples were prepared in the same manner as outlined in Section 2.5. Self-fluorescing properties of whey protein were used in order to prevent any changes in the microstructure that could be induced through staining, or covalent tagging of the molecules. A Leica TCS-SPE confocal microscope (Leica Microsystems Ltd, UK) fitted with an argon laser was used for all CLSM analysis. Protein particles were excited at 405 nm and detected at 450–500 nm. Stacked images were obtained using 0.5  $\mu\text{m}$  spacing through the gel particles under 40 $\times$  and 60 $\times$  magnification; Immersion oil (56822, Sigma–Aldrich, UK) was used to bridge the gap between coverslip and objective lens. Images were again processed using a software package (ImageJ) to extract 3D images and enhance the contrast.

## 2.7. Rheological methods

A Protein content of 9% was calculated for gel particles based on limited denaturation (Croguennec, O’Kennedy, & Mehra, 2004). However, material response for fluid gels arises through the volume occupied by the particles; thus for all rheological testing samples were prepared in volume fractions ( $\phi_{\text{FG}}$ ) from 55 to 90% on a gel particle basis.  $\phi_{\text{FG}}$  were prepared by first dispersing the fluid gel particles using an overhead stirrer. Aliquots (10 ml) were removed using a syringe and added to centrifuge tubes. The free water was removed from the system using centrifugation, Sigma 3K30 (Sigma Laborzentrifugen GmbH, Germany), at 10,000 RCF for 10 min. Relative centrifugal force and duration was determined experimentally as the point at which continued water loss ceased for all fluid gels. The supernatant was removed and 100% volume fraction obtained. Gel particles were then diluted with deionised water to give desired  $\phi_{\text{FG}}$ . Suspensions were further stored at 5  $^{\circ}\text{C}$  for 24 h before testing. All fluid gel samples were analysed at 25  $^{\circ}\text{C}$  and subjected to pre-shear ( $10\text{ s}^{-1}$  for 10 s) to ensure consistent loading conditions.

### 2.7.1. Dynamic oscillatory measurements

Frequency sweeps were conducted with a serrated 25 mm parallel plate and serrated bottom plate using a 1 mm gap. All frequency sweeps were obtained in controlled strain mode between 0.1 and 10 Hz. Strains were determined by amplitude sweeps and were set to within the linear viscoelastic region (LVR) for all samples tested (<1% strain).

### 2.7.2. Viscosity measurements

Thixotropic studies were undertaken using the same measuring system as described in Section 2.7.1. Fluid gel samples with  $\phi_{\text{FG}}$  90 were subjected to increasing shear rates from 0.01 to  $800\text{ s}^{-1}$  before decreasing the shear rate ( $800\text{--}0.01\text{ s}^{-1}$ ). This method was then repeated using a 5 min recovery period in between sweeps.

## 3. Results and discussion

### 3.1. Formulation of WPI fluid gels

#### 3.1.1. Effects of shear rate on the degree of aggregation

Viscometry was used to study the formation of 10 wt% whey protein fluid gels at native pH (~6.4). Fig. 1 shows the changes in apparent viscosity for WPI solutions at different shear rates ( $200\text{--}800\text{ s}^{-1}$ ) as the temperature was increased from 25 to 80  $^{\circ}\text{C}$  at  $1\text{ }^{\circ}\text{C min}^{-1}$  and subsequently held for 10 min. On heating, the system’s apparent viscosity slowly decreases (Arrhenius effect) until a critical temperature is reached, above which, a rapid increase in the viscosity is observed. The viscosity increases in a two-

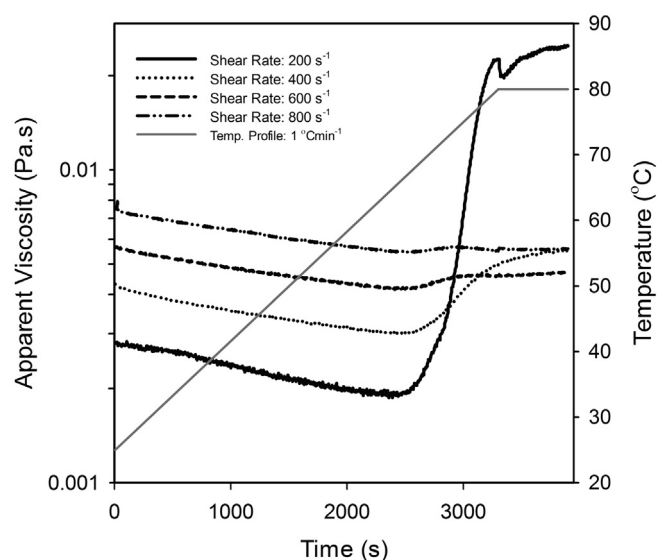


Fig. 1. Viscosity profiles for 10 wt% WPI solutions upon heating from 25  $^{\circ}\text{C}$  to 80  $^{\circ}\text{C}$  at  $1\text{ }^{\circ}\text{C min}^{-1}$  followed by subsequent holding for 10 min.

step fashion, which has previously been reported for other fluid gels such as  $\kappa$ -carrageenan (Gabriele et al., 2009), where the mechanism for particle formation was of nucleation and growth. Growth of the initial nuclei has previously been reported to occur through one of two mechanisms: enrichment from the surrounding un-gelled polymer, or particle–particle aggregation as two particles come together (coalesce) (Gabriele et al., 2009; Garrec & Norton, 2012). The formation of WPI gel particles can also be explained on the same grounds. Heat induced gelation of whey proteins is a multi-step mechanism primarily led with the unfolding of the native structure (Bauer, Hansen, & Øgendal, 1998; Cairolì, Lametti, & Bonomi, 1994; Hoffmann, Roefs, Verheul, Van Mil, & De Kruif, 1996; Sawyer, 1968). The WPI gel structure is mainly formed through the polymerisation of  $\beta$ -lactoglobulin, WPI’s major protein constituent, which initially partially denatures whilst retaining its dimeric form. With further restructuring to the secondary structure, intermolecular  $\beta$ -sheets provide points of association leading to the formation of oligomers with higher molecular mass (Lefèvre & Subirade, 2000) accompanied by an observed change in turbidity in accordance with Walkenstrom, Windhab, and Hermansson (1998). These then act as nuclei that grow via enrichment from unfolded neighbouring protein until a primary particle is formed. At this initial stage, the number and volume fraction of initial particles are mainly responsible for the increase in viscosity (Mudgal, Daubert, & Foegeding, 2011) (onset to the viscosity increase). As the onset of viscosity increase remains constant across all systems, it is suggested that the number and size of the initial particles is not affected by the shear applied (Fig. 1). This is in agreement with the current knowledge on protein denaturation/aggregation as the formation of the initial clusters occurs over a time scale of seconds (Morris, Watzky, & Finke, 2009). As the aggregation process continues the number of primary particles within the system rises until particle–particle aggregation becomes dominant. This leads to the formation of large aggregates with increased phase volume. As a result the viscosity of the system increases as expected for highly flocculated suspensions (Barnes, 1994; Buscall et al., 1987; Chung et al., 2014; Goodwin & Reynolds, 1998). The application of a shear field during gelation is therefore the main factor responsible for limited aggregate growth preventing aggregation spanning across the entire volume.



Correspondingly, the viscosity change throughout the sol–gel transition decreases with increasing shear rates from 200 to 800 s<sup>−1</sup>, as shown in Fig. 1, as particle–particle aggregation becomes increasingly hindered. At the later stage of the process strengthening of the matrix through covalent bonding i.e. disulphide bonds occurs until a final aggregate is attained (Le Bon, Nicolai, & Durand, 1999).

Confocal laser scanning micrographs of an individual WPI aggregate and also single particles are shown in Fig. 2a and b respectively. Fluid gels particles prepared at 200 s<sup>−1</sup> were primarily large and highly aggregated, seeming to have formed through interconnected particles, resulting in a three-dimensional network as previously described. However, by increasing the shear rate to 800 s<sup>−1</sup> whilst undergoing the sol–gel transition, it was observed that applied separation was sufficient to prevent any secondary aggregation, restricting the final aggregates to the size of the primary particles (Fig. 2b). This again reinforced the hypothesis that growth occurs via the bonding of primary particles to form larger aggregates that grow to a size dictated by the applied shear field.

### 3.1.2. Effects of heating rate

The effect of applied heating rate on the production of WPI fluid gels is depicted in Fig. 3. Here the apparent viscosity profiles of 10 wt% WPI solutions were measured on heating to 80 °C at 10 °C min<sup>−1</sup> (Fig. 3a). The obtained data revealed that as the heating rate increased the overall viscosities of the systems increased when compared to those prepared at 1 °C min<sup>−1</sup>. It is suggested that a higher degree of aggregation occurs at higher heating rates, thus increasing the effective volume of the final aggregates and consequently the viscosity of the suspensions (Genovese, 2012).

Above the critical gelling concentration nucleation is no longer the rate-limiting step for aggregation, thus larger aggregates can rapidly form resulting in higher viscosities (Mudgal, Daubert, & Foegeding, 2009; Mudgal et al., 2011; Walkenstrom et al., 1998). As such, the relationship between viscosity and degree of aggregation was applied to study the rate of aggregation (Fig. 3b) in relation to both heating and shear rates, using apparent viscosity data obtained throughout the gelation process. The rate of aggregation ( $d\alpha/dt$ ), was calculated as:

$$d\alpha = \frac{\eta_i - \eta_{i-1}}{\eta_0} \quad (1)$$

$$\text{Rate of Aggregation} = \left( \frac{d\alpha}{dt} \right)_i \quad (2)$$

where  $\eta_i$  and  $\eta_{i-1}$  are the viscosity at time  $t_i$  and  $t_{i-1}$  respectively, and  $\eta_0$  is the initial viscosity. Corresponding values of  $t_i$  and  $t_{i-1}$  were determined from the linear region of the viscosity curve after the onset of viscosity had been reached. It was observed that the rate of aggregation was dependent on both the shear rate and the heating rate applied to the system. Fig. 3b clearly shows that increasing the shear rate during the sol–gel transition, slows the rate at which aggregates form across all heating rates studied: as the imposed shear hinders the denatured protein from coming into contact and bonding.

On the other hand, as the heating rate increases, the rate of aggregation increases (Fig. 3b), owing to the temperature dependence of aggregate growth being purely kinetic (Le Bon et al., 1999). Therefore, the number of primary particles that interact to form an aggregate is also expected to increase following an equation originally proposed by Berli, Deiber, and Añón (1999):

$$N = 1 + k(t - t_0) \quad (3)$$

where  $N$  is the number of primary particles,  $t_0$  is the initial time at which  $N = 1$ , and  $k$  is the aggregation rate constant. Hence, as  $K$  increases, due to higher heating rates, the number of primary particles coming together also increases, resulting in the formation of larger aggregates. This explains the difference in viscosity profiles observed between solutions heated at 10 °C min<sup>−1</sup> and those heated at 1 °C min<sup>−1</sup>. However as suggested by Gabriele et al. (2009) increasing the applied heating rate would decrease the processing time, as a consequence the system experiences the given shear for less time, thus decreasing the likelihood that larger aggregates are not broken down.

### 3.1.3. Qualitative model for particle formation

Size distributions for WPI gel particles made at varying shear regimes and two heating rates 1 and 10 °C min<sup>−1</sup> are shown in Fig. 4a and b respectively. It was observed that fluid gels produced in this manner were mono-modal, with the exception of samples prepared at the lowest shear rate, where a small shoulder can be observed. The presence of this shoulder may be due to large aggregates becoming trapped in the proximity of the vane geometry during processing, thus avoiding the shear field (Fig. 4a). As shear

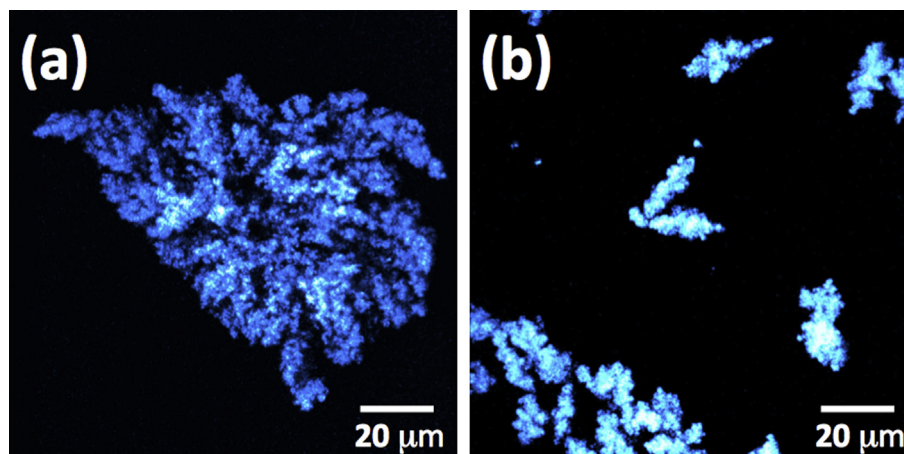
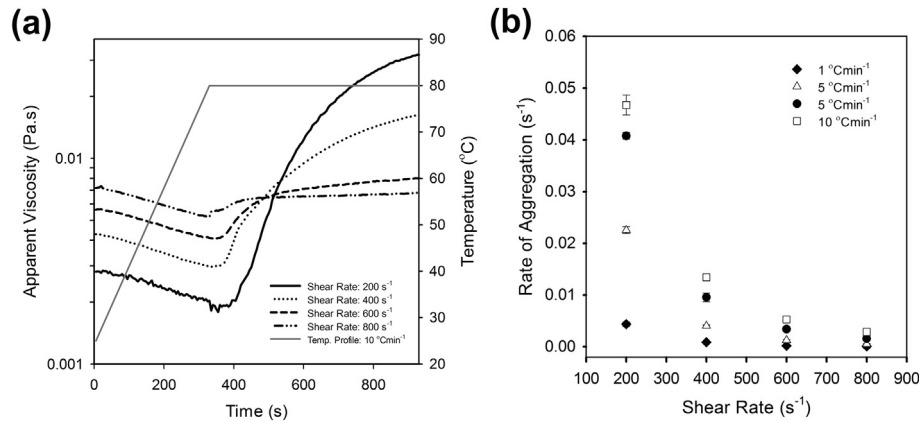
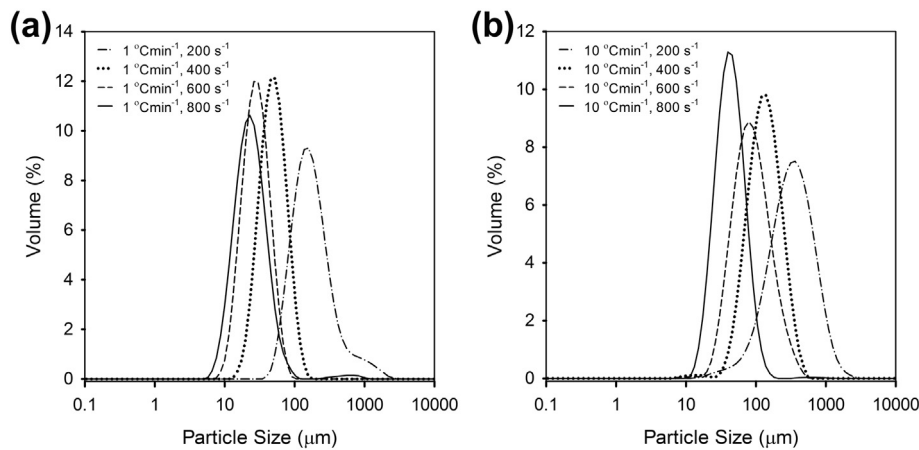


Fig. 2. Diluted WPI fluid gel particles (1:4) viewed under confocal laser scanning microscopy: samples were excited at 405 nm using 40× magnification. Particles were prepared at 1 °C min<sup>−1</sup> at 200 s<sup>−1</sup> (a) and 800 s<sup>−1</sup> (b) (Scale bar shows 20 μm).



**Fig. 3.** (a) Viscosity profiles observed during sheared heating from 25 °C to 80 °C at 10 °C min<sup>-1</sup> and (b) the rate of protein aggregation at varying shear rates as a function of the rate of applied heat.

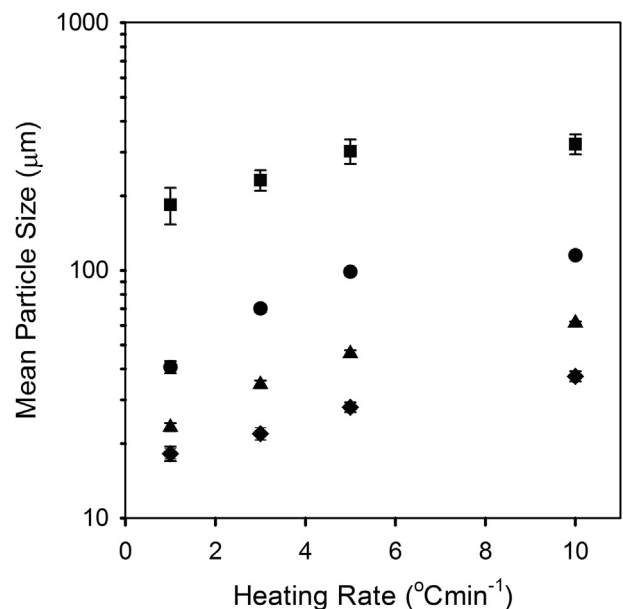


**Fig. 4.** SLS size distributions obtained for WPI fluid gels prepared using varying shear rates (200–800 s<sup>-1</sup>) at 1 °C min<sup>-1</sup> (a) and 10 °C min<sup>-1</sup> (b).

increased the distributions shifted towards lower particle sizes as growth during the gelation process becomes increasingly limited by the imposed shear. Distributions for particles prepared at 10 °C min<sup>-1</sup> have been shown in Fig. 4b. Again mono-modal peaks were observed; however, aggregates produced at higher heating rates were larger in size as predicted by Eq. (3).

Mean sizes for all WPI fluid gel particles obtained at varying shear and thermal histories have been shown in Fig. 5. The data obtained showed a relationship between the size of the WPI particles and shear rate employed during the process; with increasing shear forming smaller aggregates. Particle size also shifted towards higher values as the heating rate increased, for all applied shear rates, as earlier ascribed to faster aggregation kinetics and resulting increase in particle–particle aggregation. To this end, it can be seen that particle size, and thus the degree of aggregation, is dictated by both the shear exerted, and heating rate applied to the system. This therefore shows that the rate of aggregation is a function of the two parameters and should be accounted for when considering “*k*” (Eq. (3)).

It is clear that the reduction in particle size as a function of applied shear rate is highly dependent on the heating rate adopted in the formation process; shown by particles made at 1 °C min<sup>-1</sup> becoming further reduced in size than those prepared at 10 °C min<sup>-1</sup> when compared at the same shear rate. This is clear evidence for a change in the aggregate formation mechanism. It is suggested that in the initial stages of aggregate formation,



**Fig. 5.** Mean particle size (*D*[4,3]) as obtained by SLS for WPI fluid gels prepared at a range of heating rates as a function of processing shear rate: 200 s<sup>-1</sup> (■), 400 s<sup>-1</sup> (●), 600 s<sup>-1</sup> (▲) and 800 s<sup>-1</sup> (◆).

relatively weak hydrophobic interactions hold the primary particles together. At this stage the shear-induced aggregate breakdown is more effective than in the later stages of formation where particles become more firmly structured through covalent bonding. Therefore, when energy is applied to the system at a faster rate (higher heating rates), aggregates are more rapidly formed that quickly becoming resistant to shear breakdown. Thus at  $10\text{ }^{\circ}\text{C min}^{-1}$  aggregates are formed within the shear field, whereas at  $1\text{ }^{\circ}\text{C min}^{-1}$  initial larger aggregates are formed that become subsequently broken down, resulting in smaller final sizes.

As the size of the aggregates increases through interlinking particles, system viscosity would be expected to increase in agreement with all data reported on highly aggregated suspensions (Barnes, 1994; Genovese, 2012; Goodwin & Reynolds, 1998). Fig. 6 shows the change in relative system viscosity across all shear and thermal histories calculated using Eq. (4).

$$\Delta\eta = \frac{\eta_{\text{final}} - \eta_{\text{initial}}}{\eta_{\text{initial}}} \quad (4)$$

Neglecting effects posed by the anisotropic morphology of the particle and subsequent orientation in flow, changes in the shear field result in the limitation of aggregation within the dispersed phase. Following the general dependence of viscosity on the dispersed phase volume fraction (Krieger & Dougherty, 1959), the viscosity of an aggregated suspension is assumed to be based primarily on the effective volume of the disperse phase, thus depending on the degree of aggregation (Barnes, 2000; Genovese, 2012). As explained earlier the effective volume fraction depends on the mechanism of aggregation leading to the number of aggregates per resulting particle. This can be clearly seen in Fig. 6 for suspensions prepared at 400, 600 and  $800\text{ s}^{-1}$ , where the change in system viscosity mirrors the increase in particle size (Fig. 5). On the other hand suspensions prepared at  $200\text{ s}^{-1}$  exhibit similar viscosity changes (over all heating rates), despite the large difference in particle size. It is suggested that for such large aggregates the effective volume fraction is close to the maximum packing fraction (highly concentrated regime), where flow is not only characterised

by size, but also by particles moving, “squeezing”, past each other. The bulk viscosity thus becomes a function of particle deformability and packing similarly to the behaviour of highly “packed” emulsions (Barnes, 1994). From this it is reasonable to assume that the deformability of the aggregate is a function of its size, with larger more aggregated particles being more deformable than smaller rigid ones (Barnes, 1994). This in turn would explain the constant viscosity observed for fluid gels made at  $200\text{ s}^{-1}$ . From the above results we conclude that aggregation of self-similar primary particles to form large aggregates represents the mechanism for WPI fluid gels. Further building on the last statement, aggregate formation through this process can either occur within the shear field, or as gel formation and subsequent breakdown.

### 3.2. Fluid gel material properties

#### 3.2.1. Small deformation rheology of fluid gels

To further probe particle mechanical properties, WPI fluid gels were characterised under small deformation rheology. Fig. 7a shows frequency sweeps obtained for fluid gels prepared at different shear rates at a given volume fraction ( $\phi_{\text{FG}}$ ) of 90%. The mechanical spectra for both gels is typical of an interconnected structure where elastic modulus ( $G'$ ) dominates the viscous component ( $G''$ ) as described by Ross-Murphy (1994), with both moduli only slightly dependant on frequency as previously reported for  $\kappa$ -carrageenan fluid gels (Garrec & Norton, 2012). Fig. 7b shows amplitude sweeps for the same fluid gel samples. The linear viscoelastic region extends to a critical strain above which the material begins to flow. The critical strain required to initiate flow decreased as the shear rate used in the preparation of the fluid gels increased. Although WPI fluid gels made at higher heating rates are characterised by a higher degree of aggregation, the ability of the aggregates to deform may also play a role at such a high strain. Large aggregates with a more open network structure are expected to be softer, as such will deform under applied shear before any flow is induced. Deformation of soft particles could lead to a larger contact area and greater friction (Yoon, Singh, Oh, & Kong, 2005), in turn increasing their maximum strain within the linear viscoelastic region. Once flowing, particles produced at lower shear rates showed a lower dependence on strain implying greater interparticle structuring (Garrec et al., 2013).

Fig. 8 shows that the elastic modulus ( $G'$ ) is highly dependent on particle phase volume ( $\phi_{\text{FG}}$ ) where  $G'$  is reported for different volume fractions. Elasticity arises through the close packing of particles above a critical phase volume, 0.55. Fig. 8 clearly shows that the elasticity of the suspensions is a function of both the shear and heating rates used in the formation process. Fluid gels made at  $10\text{ }^{\circ}\text{C min}^{-1}$  exhibit higher elastic moduli than those prepared at  $1\text{ }^{\circ}\text{C min}^{-1}$  since higher heating rates favour particle–particle interactions. On the other hand shear induced breakdown will limit the extent of particle–particle interactions, which explains the lower storage modulus for particles made at  $800\text{ s}^{-1}$ . Although suspension elasticity arises primarily through interactions between particles, the size of the aggregates should also be considered to negate against effects such as deformability and packing (Barnes, 1994). To this end, the extent of interactions between aggregates was further studied by comparing fluid gels of similar particle size (Fig. 9). Although particles were of similar size (ca.  $40\text{ }\mu\text{m}$ ) it was observed that the two showed different elastic moduli. Again, elasticity was predominantly dictated by the level of shear applied during the formation process with fluid gels prepared at  $400\text{ s}^{-1}$  having a higher elastic modulus than those made at  $600\text{ s}^{-1}$ . This indicates that aggregates formed where size is limited more greatly through shear separation, interact with each other to a lower extent.

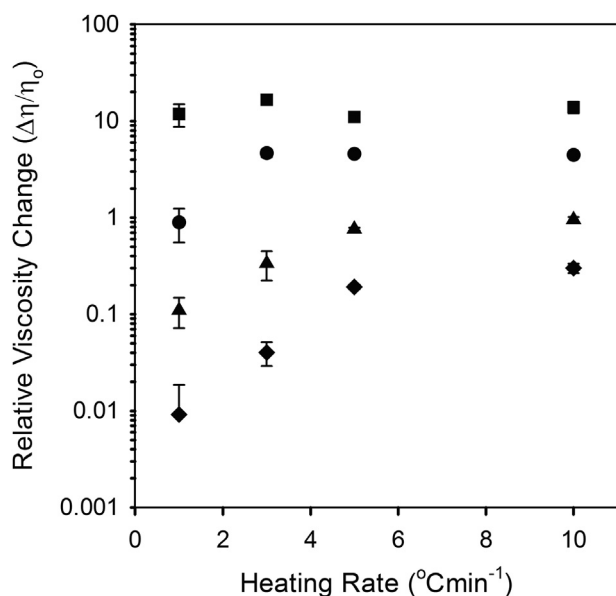
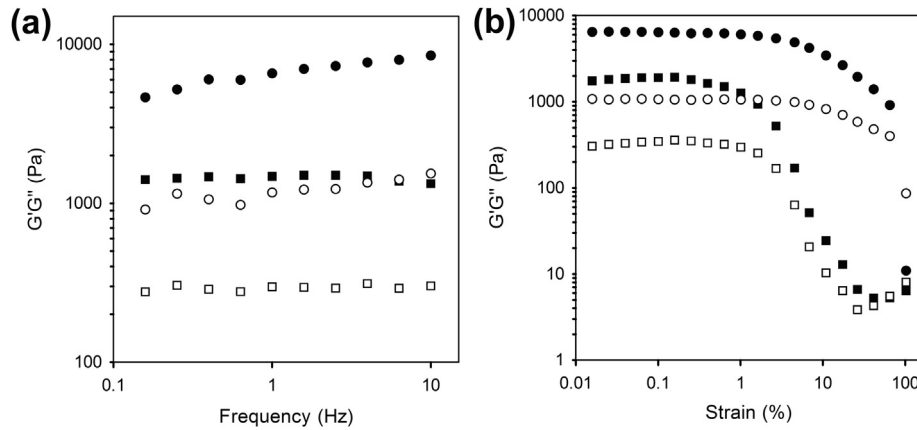


Fig. 6. Relative change in viscosity obtained from fluid gel viscosity profiles. Fluid gels were prepared at varying heating rates ( $1\text{--}10\text{ }^{\circ}\text{C min}^{-1}$ ) as a function of shear rate  $200\text{ s}^{-1}$  (■),  $400\text{ s}^{-1}$  (●),  $600\text{ s}^{-1}$  (▲) and  $800\text{ s}^{-1}$  (◆).



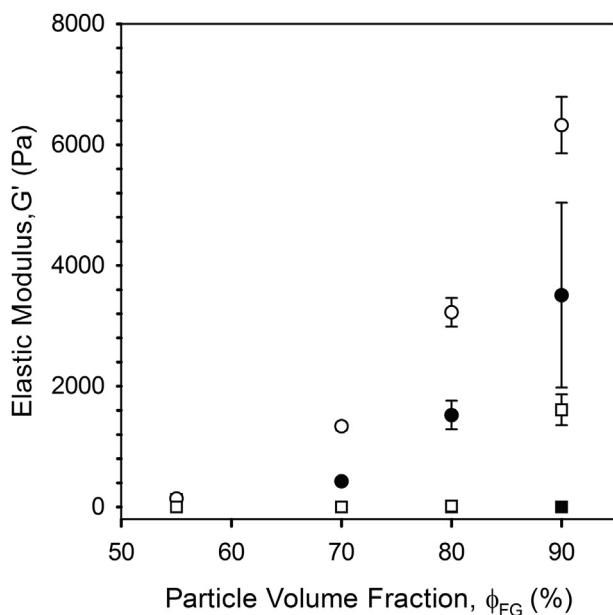
**Fig. 7.** (a) Amplitude and (b) frequency sweeps for fluid gels  $\phi_{FG}90$  prepared at  $200\text{ s}^{-1}$  (●, ○) and  $800\text{ s}^{-1}$  (■, □),  $10\text{ }^{\circ}\text{C min}^{-1}$ . Closed markers denote  $G'$  and open,  $G''$ . Amplitude sweep was obtained at 1 Hz and frequency sweep at 0.05% strain and show the average of three repeats.

### 3.2.2. Fluid gel flow behaviour

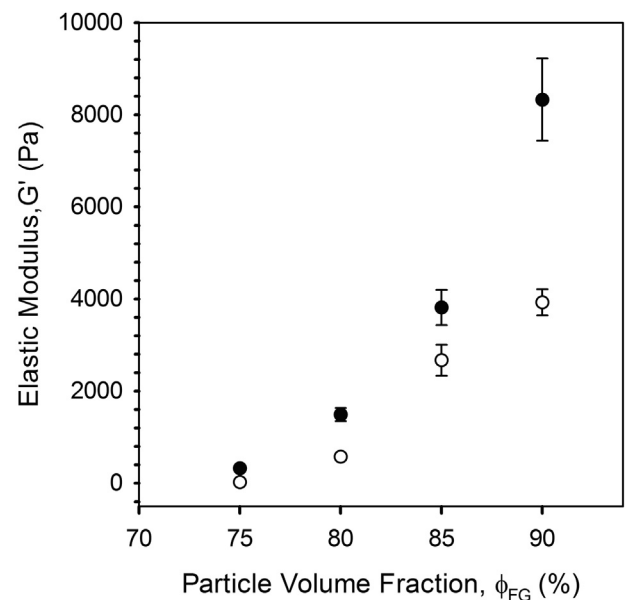
Flow behaviours of WPI shear gels formed at  $1\text{ }^{\circ}\text{C min}^{-1}$  are shown in Fig. 10a. Again, a comparison is made across suspensions with the same phase volume (90%). Fluid gels exhibit characteristic shear thinning behaviour typical of flocculated suspensions (Berli & Quemada, 2000; Quemada, 1998). At low shear rates systems show an apparent shear thickening until a small Newtonian plateau is reached; which increases as a function of the processing parameters. It was observed that smaller aggregates had lower viscosities than their counterparts prepared at lower shear rates. This clearly demonstrates that for the same degree of space filling, the more aggregated particles produce a higher viscosity. Viscosities at the first Newtonian plateau ( $0.03\text{ s}^{-1}$  for all samples) for WPI fluid gels prepared at different shear and heating rates are compared in Fig. 10b. Increasing the shear rate during production led to a decrease in the suspension viscosity at both heating rates, again due to the lower degree of particle–particle interactions (Fig. 10b).

With increasing shear rate the viscosity decreases following shear-thinning behaviour. The nonlinearity in the systems is either due to alignment of suspended particles in the direction of flow or to the breakdown of the aggregates (Quemada, 1998). For this reason sample data is presented for both the upward and downward shear sweeps. The presence of hysteresis revealed a thixotropic behaviour arising from aggregate breakdown that led to an overall change in the phase volume; as such the viscosity decreases.

A second sweep separated by a 5 min recovery period was used to explore the reduction in viscosity with shear. After the recovery time it was observed that the second sweep followed a similar profile to the decrease in shear observed in the first sweep, shown in Fig. 11a. This suggested that no recovery of the system occurred within this time frame, inferring that the lowering in viscosity observed in the first sweep was caused by permanent damage to the aggregates: this was confirmed by SLS data that showed a shift in the size distributions from 184 to 156  $\mu\text{m}$ . The degree of thixotropy (calculated as the area within the hysteresis loop) was compared across all fluid gels for different processing conditions:

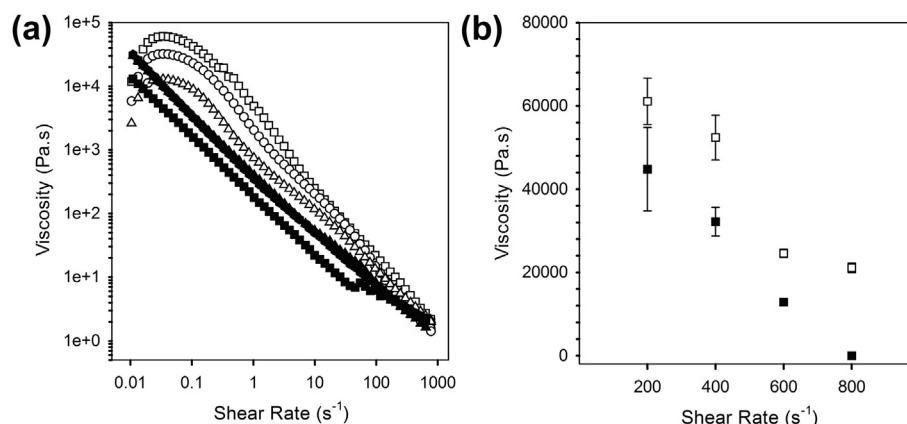


**Fig. 8.** Small deformation oscillatory data obtained for WPI fluid gels prepared at  $200\text{ s}^{-1}$  (●, ○) and  $800\text{ s}^{-1}$  (■, □). Solid markers represent fluid gels formulated at  $1\text{ }^{\circ}\text{C min}^{-1}$  and open  $10\text{ }^{\circ}\text{C min}^{-1}$ . Values of  $G'$  were obtained at 1 Hz.

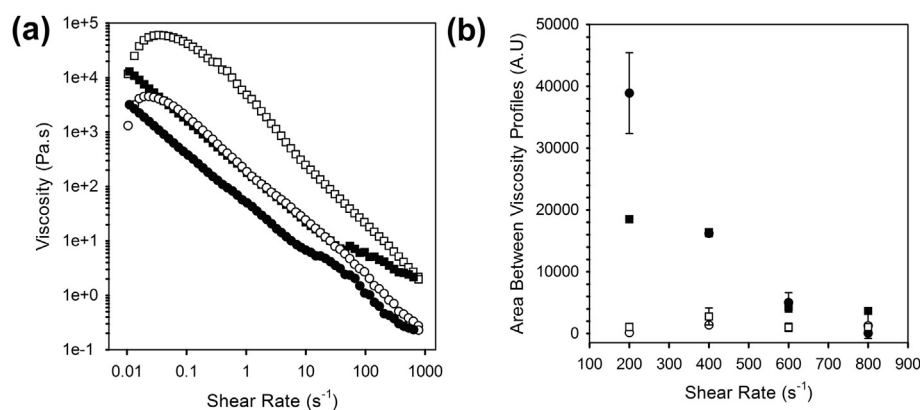


**Fig. 9.** Elastic moduli ( $G'$ ) plotted against volume fraction ( $\phi_{FG}$ ) for fluid gel particles of similar size (ca.  $30\text{ }\mu\text{m}$ ). Particles were prepared at  $400\text{ s}^{-1}$  ( $1\text{ }^{\circ}\text{C min}^{-1}$ ) (●) and  $600\text{ s}^{-1}$  ( $5\text{ }^{\circ}\text{C min}^{-1}$ ) (○).





**Fig. 10.** (a) Viscosity curves showing thixotropic behaviour for fluid gels prepared at 1 °C min<sup>-1</sup>, 200 s<sup>-1</sup> (□, ■), 400 s<sup>-1</sup> (○, ●) and 600 s<sup>-1</sup> (Δ, ▲)  $\phi$ FG90. (Unfilled symbols denote ramp up and filled ramp down.). Data shows the average of three repeats. (b) Fluid gel viscosities at the first Newtonian plateau (0.03 s<sup>-1</sup>) having been prepared using varying shear rates at 1 °C min<sup>-1</sup> (■) and 10 °C min<sup>-1</sup> (□).



**Fig. 11.** (a) Flow curves for WPI fluid gels (prepared at 200 s<sup>-1</sup>, 1 °C min<sup>-1</sup>) sweep 1 (□, ■) and sweep 2 (○, ●). A 5 min recovery period was held between sweeps. (Open symbols denote increasing shear rates, filled denote decreasing shear rate.). Data shows the average of three repeats. (b) Thixotropic analysis for WPI fluid gels formulated at 1 °C min<sup>-1</sup> (●, ○) and 10 °C min<sup>-1</sup> (■, □) as a function of processing shear rate (closed markers represent sweep 1 and open, sweep 2).

heating and shear rates (Fig. 11b). The evaluation of the hysteresis area revealed that the structural breakdown occurs mainly during the first ramp and is more apparent for fluid gels made at low shear rates 200 s<sup>-1</sup>. This infers that large aggregates are more easily disrupted compared to smaller ones due to a higher probability of inherent weak spots.

#### 4. Conclusions

This study has shown that fluid gels can be prepared using whey protein under controlled shear and thermal conditions. A qualitative model for the formation of WPI gel particles and their dependence on processing conditions has also been presented. This model is primarily based on the general understanding of growth and the aggregation process. Firstly, unfolded proteins interact to form primary particles. In the second stage these primary particles move into contact with each other and are held together by relatively weak forces. Ultimately, the particles become more firmly bound as result of covalent bonding, i.e. disulphide bonds, forming secondary aggregates/final particles. It is in this second stage that structural organisation can be controlled through manipulating the rate of aggregation. Fast heating rates will promote particles that interact quickly becoming rapidly resistant to shear and as such large aggregates are produced. On the other hand low heating rates or increasing shear rates lower the rate of aggregation slowing the

strengthening stage, causing particles to be more affected by the flow and therefore smaller aggregates or even single resulting particles are produced. The bulk properties of the suspensions are ultimately governed by the interaction of the final aggregates once in close enough proximity (high volume fraction). The elasticity of the suspensions was shown to strongly depend on the degree of shear applied during formation with lower shear rates showing increased aggregate–aggregate interacting. The flow behaviour of the suspensions is characterized in terms of a reduced phase volume, where it is inferred that particles occupy different effective volumes as a function of the degree of aggregation. The suspensions showed marked shear thinning behaviour depending upon the breakdown of the aggregates. Finally this study has shown that whey protein can be used to prepare fluid gels that are both thermally stable and nutritionally desirable.

#### Acknowledgements

The authors would like to thank EPSRC (EP/G036713/1), Kerry Group, and also Birmingham Science City: Innovative Uses for Advanced Materials in the Modern World (West Midlands Centre for Advanced Materials Project 2), with support from Advantage West Midlands (AWM) and part funded by the European Regional Development Fund (ERDF), for the use of the confocal microscope used in this research.

## References

- Adams, S., Frith, W. J., & Stokes, J. R. (2004). Influence of particle modulus on the rheological properties of agar microgel suspensions. *Journal of Rheology*, 48(6), 1195.
- Altmann, N., Cooper-White, J. J., Dunstan, D. E., & Stokes, J. R. (2004). Strong through to weak 'sheared' gels. *Journal of Non-Newtonian Fluid Mechanics*, 124(1–3), 129–136.
- Barnes, H. A. (1994). Rheology of emulsions — a review. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 91, 89–95.
- Barnes, H. A. (2000). *A handbook of elementary rheology*. University of Wales, Institute of Non-Newtonian Fluid Mechanics.
- Bauer, R., Hansen, S., & Øgødal, L. (1998). Detection of intermediate oligomers, important for the formation of heat aggregates of  $\beta$ -lactoglobulin. *International Dairy Journal*, 8(2), 105–112.
- Berli, C. L. A., Deiber, J. A., & Anón, M. C. (1999). Heat-induced Phenomena in Soy protein suspensions. Rheometric data and Theoretical Interpretation. *Journal of Agricultural and Food Chemistry*, 47(3), 893–900.
- Berli, C. L. A., & Quemada, D. (2000). Rheological modeling of microgel suspensions involving solid–liquid transition. *Langmuir*, 16(21), 7968–7974.
- Buscall, R., Mills, P. D. A., Stewart, R. F., Sutton, D., White, L. R., & Yates, G. E. (1987). The rheology of strongly-flocculated suspensions. *Journal of Non-Newtonian Fluid Mechanics*, 24(2), 183–202.
- Cairolì, S., Lametti, S., & Bonomi, F. (1994). Reversible and irreversible modifications of  $\beta$ -lactoglobulin upon exposure to heat. *Journal of Protein Chemistry*, 13(3), 347–354.
- Chung, C., Degner, B., & McClements, D. J. (2014). Development of reduced-calorie foods: microparticulated whey proteins as fat mimetics in semi-solid food emulsions. *Food Research International*, 56(0), 136–145.
- Croguennec, T., O'Kennedy, B. T., & Mehra, R. (2004). Heat-induced denaturation/aggregation of  $\beta$ -lactoglobulin A and B: kinetics of the first intermediates formed. *International Dairy Journal*, 14(5), 399–409.
- Dissanayake, M., Kelly, A. L., & Vasiljevic, T. (2010). Gelling properties of microparticulated whey proteins. *J Agric Food Chem*, 58(11), 6825–6832.
- Dissanayake, M., & Vasiljevic, T. (2009). Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. *J Dairy Sci*, 92(4), 1387–1397.
- Fernández Farrés, I., Douaire, M., & Norton, I. T. (2013). Rheology and tribological properties of Ca-alginate fluid gels produced by diffusion-controlled method. *Food Hydrocolloids*, 32(1), 115–122.
- Fernández Farrés, I., Moakes, R. J. A., & Norton, I. T. (2014). Designing biopolymer fluid gels: a microstructural approach. *Food Hydrocolloids*, 42(Part 3), 362–372.
- Gabriele, A., Spyropoulos, F., & Norton, I. T. (2009). Kinetic study of fluid gel formation and viscoelastic response with kappa-carrageenan. *Food Hydrocolloids*, 23(8), 2054–2061.
- Gabriele, A., Spyropoulos, F., & Norton, I. T. (2010). A conceptual model for fluid gel lubrication. *Soft Matter*, 6(17), 4205–4213.
- Garrec, D. A., Guthrie, B., & Norton, I. T. (2013). Kappa carrageenan fluid gel material properties. Part 1: rheology. *Food Hydrocolloids*, 33(1), 151–159.
- Garrec, D. A., & Norton, I. T. (2012). Understanding fluid gel formation and properties. *Journal of Food Engineering*, 112(3), 175–182.
- Genovese, D. B. (2012). Shear rheology of hard-sphere, dispersed, and aggregated suspensions, and filler-matrix composites. *Advanced Colloid and Interface Science*, 171–172, 1–16.
- Goodwin, J. W., & Reynolds, P. A. (1998). The rheology of flocculated suspensions. *Current Opinion in Colloid and Interface Science*, 3(4), 401–407.
- Hoffmann, M. A. M., Roefs, S. P. F. M., Verheul, M., Van Mil, P. J. J. M., & De Kruif, C. G. (1996). Aggregation of beta-lactoglobulin studied by in situ light scattering. *Journal of Dairy Research*, 63(3), 423–440.
- Krieger, I. M., & Dougherty, T. J. (1959). A mechanism for non-newtonian flow in suspensions of rigid spheres. *Journal of Rheology*, 3(1), 137–152.
- Le Bon, C., Nicolai, T., & Durand, D. (1999). Growth and structure of aggregates of heat-denatured beta-Lactoglobulin. *International Journal of Food Science and Technology*, 34(5–6), 451–465.
- Lefèvre, T., & Subirade, M. (2000). Molecular differences in the formation and structure of fine-stranded and particulate beta-lactoglobulin gels. *Biopolymers*, 54(7), 578–586.
- McClements, D. J., & Keogh, M. K. (1995). Physical properties of cold-setting gels formed from heat-denatured whey protein isolate. *Journal of the Science of Food and Agriculture*, 69(1), 7–14.
- Meza, B., Verdini, R., & Rubiolo, A. (2010). Viscoelastic behavior during the ripening of a commercial low-fat soft cheese. *Dairy Science & Technology*, 90(5), 589–599.
- Morris, A. M., Watzky, M. A., & Finke, R. G. (2009). Protein aggregation kinetics, mechanism, and curve-fitting: a review of the literature. *Biochim Biophys Acta*, 1794(3), 375–397.
- Mudgal, P., Daubert, C. R., & Foegeding, E. A. (2009). Cold-set thickening mechanism of  $\beta$ -lactoglobulin at low pH: concentration effects. *Food Hydrocolloids*, 23(7), 1762–1770.
- Mudgal, P., Daubert, C. R., & Foegeding, E. A. (2011). Kinetic study of  $\beta$ -lactoglobulin thermal aggregation at low pH. *Journal of Food Engineering*, 106(2), 159–165.
- Nicolai, T., Britten, M., & Schmitt, C. (2011).  $\beta$ -lactoglobulin and WPI aggregates: formation, structure and applications. *Food Hydrocolloids*, 25(8), 1945–1962.
- Norton, I. T., Jarvis, D. A., & Foster, T. J. (1999). A molecular model for the formation and properties of fluid gels. *International Journal of Biological Macromolecules*, 26(4), 255–261.
- Norton, J. E., & Norton, I. T. (2010). Designer colloids—towards healthy everyday foods? *Soft Matter*, 6(16), 3735–3742.
- Quemada, D. (1998). Rheological modelling of complex fluids. I. The concept of effective volume fraction revisited. *The European Physical Journal of Applied Physics*, 1(1), 119–127.
- Ross-Murphy, S. B. (1994). Rheological characterization of polymer gels and networks. *Polymer Gels and Networks*, 2(3–4), 229–237.
- Sawyer, W. H. (1968). Heat denaturation of bovine  $\beta$ -lactoglobulins and relevance of disulfide aggregation. *Journal of Dairy Science*, 51(3), 323–329.
- Singer, N. S., Yamamoto, S., & Latella, J. (1988). In *Protein product base* (Vol. 4734287). John LaBatt Limited.
- Sullo, A., Watson, R. L., & Norton, I. T. (2014). Design of colloidal foods for healthier diets. In *Gums and stabilisers for the food industry 17: The changing face of food manufacture: The role of hydrocolloids* (pp. 287–302). The Royal Society of Chemistry.
- Torres, I. C., Janhøj, T., Mikkelsen, B. Ø., & Ipsen, R. (2011). Effect of microparticulated whey protein with varying content of denatured protein on the rheological and sensory characteristics of low-fat yoghurt. *International Dairy Journal*, 21(9), 645–655.
- Walkenstrom, P., Windhab, E., & Hermansson, A.-M. (1998). Shear-induced structuring of particulate whey protein gels. *Food Hydrocolloids*, 12, 459–468.
- Yoon, E.-S., Singh, R. A., Oh, H.-J., & Kong, H. (2005). The effect of contact area on nano/micro-scale friction. *Wear*, 259(7–12), 1424–1431.